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Extraction and Gas-Liquid Chromatographic Analysis of Chlorphoxim in Water and Fish

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Chlorphoxim (2-chloro-α-{{(diethoxyphosphinothioyl)oxy}imino} benzeneacetonitrile) has been shown to be effective against the larval stages of Simulium damnosum (blackfly), the insect vector of human onchocerciasis in Africa (LE BERRE et al. 1972). It is also effective against adult mosquitoes (HUDSON & OBUDHO 1972) and agricultural insects (DAY & CROSBY 1972, HARRIS & SVIC 1970). With more widespread use, efficient methods for analysis of environmental residues will be needed.

Two gas chromatographic methods for the analysis of chlorphoxim have been reported. The first (LEUCK & BOWMAN 1973) was a method for direct GLC analysis of chlorphoxim after it had been separated from its oxygen analog on a silica gel column. DALE, et al. (1976) found chlorphoxim to be unstable and the GLC peak not well defined under the conditions described for direct analysis and as a result developed and reported the second method. The latter method is based upon the in-block methylation of chlorphoxim with trimethylanilinium hydroxide (TMAH) and measurement of the amount of O,O-diethyl O-methyl phosphorothioate (DEMTP) formed. In the course of work with this method, it was observed that when samples and pure standards containing high concentrations of chlorphoxim were injected into the gas chromatograph under the conditions described, three trialkyl phosphates were formed. Two of these, DEMTP and O,O-diethyl S-methyl phosphorothioate were reported by DALE, et al. (1976). The third compound emerged between the two named above and interfered somewhat with DEMTP. Although this third compound has not been identified, since it was produced upon injection of a pure chlorphoxim solution, investigations were begun to find a more efficient means of conversion of chlorphoxim to DEMTP.

Use of alcoholic hydroxide solutions for purposes of derivatization of organophosphorus pesticides has been reported by MOYE (1973) and CHURCHILL (1978). Using the basic principle of transesterification by methoxide ion, a gas chromatographic method has been developed for the analysis of chlorphoxim which increases the efficiency of conversion to DEMTP by eliminating undesirable side reactions and thus reducing the time required for GLC analysis to about 3 min. The proposed method has the further advantage of allowing analysis of fish extracts without additional cleanup.

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EXPERIMENTAL

Apparatus

Gas chromatograph equipped with flame photometric detector; 1.5 m x 4 mm i.d. stainless steel column. The column packing was prepared by combining 10 g of 5% OV-225 on 100/120 mesh Chromosorb W (HP) with 5 g of 7.5% OV-275 on 100/120 mesh Chromosorb W (HP). The following conditions were maintained: Inlet and outlet block at 195°C, column at 150°C, FPD detector at 160°C; nitrogen carrier gas at 107 mL/min.

Reagents

1) Chlorphoxim. Chemagro Corp. Kansas City, MO 64120.* Recrystallized from benzene-hexane (1 + 1): mp 65.8°C.

2) DEMTP. Prepared as described by DALE et al. (1976).

Preparation of standards

1) Chlorphoxim. An acetone solution containing 500 µg/mL was prepared. Serial dilutions were then prepared in methanol to yield a chlorphoxim standard containing 10 ng/µL. Working standards of chlorphoxim were prepared at the appropriate time in 0.05 M KOH in methanol. These standards ranged in concentration from 0.1 to 1.0 ng/µL.

Using the conditions described above, 1-5 µL of each standard was injected into the gas chromatograph. Quantitation was by peak height; standard curve was linear for the concentration range used.

2) DEMTP. A methanol solution of ca. 1.0 mg/mL was prepared. Serial dilutions were then prepared in methanol to yield working standards of DEMTP containing 0.1 to 1.0 ng/µL.

Quantitation was by peak height; standard curve was linear over the concentration range used.

Efficiency of conversion of chlorphoxim to DEMTP

MILES and DALE (1978) reported that the inlet block conversion of chlorphoxim to DEMTP in 0.01 M TMAH was 66% efficient. DALE et al. (1976) found that the maximum overall efficiency of conversion of chlorphoxim to DEMTP was 90% and occurred when chlorphoxim was allowed to stand in 0.01 M TMAH for 24 h at room temperature prior to GLC analysis.

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* Mention of commercial sources does not constitute endorsement by the Public Health Service or the U.S. Department of HEW.

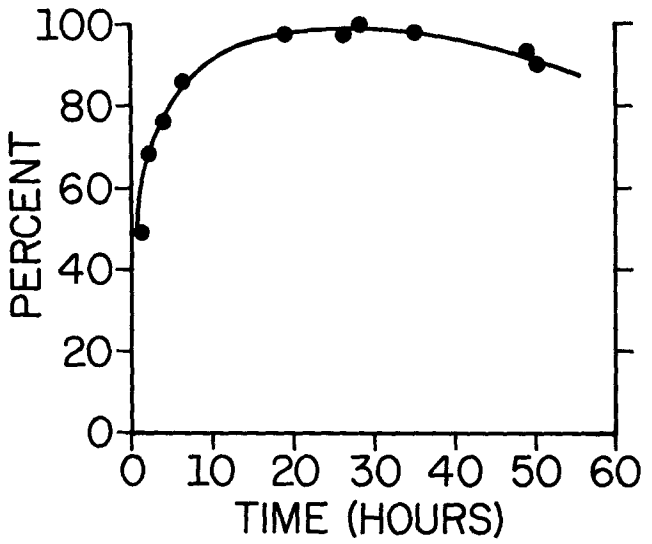

Figure 1. Efficiency of conversion of chlorphoxim to DEMTP with respect to time at room temperature.

Efficiency of conversion of chlorphoxim to DEMTP was also found to vary with time in 0.05 M KOH in methanol prior to GLC analysis. Solutions of standard chlorphoxim were prepared in 0.05 M KOH in methanol, allowed to stand at room temperature, and subjected to GLC analysis over a period of 50 h. The efficiency of transesterification was determined as described in the above time study. Maximum efficiency occurred when samples were allowed to stand between 19 and 35 h prior to injection into the gas chromatograph (Fig. 1). These results show that methanolic KOH must be added to chlorphoxim standards at the same time it is added to the unknown samples. If one is willing to accept an efficiency of 80%, samples may be analyzed within 5 to 6 h after the addition of methanolic KOH.

To determine the effect of KOH concentration, standard chlorphoxim samples were prepared in concentrations of KOH in methanol ranging from 0.001 to 0.115 M and injected into the gas chromatograph under the conditions described above after standing at room temperature overnight. The maximum efficiency of conversion of chlorphoxim to DEMTP was 98 ± 4% and occurred in concentrations of KOH methanol ranging from 0.04 to 0.08 M (Fig. 2).

Under the conditions described above, no artificial enhancement of peaks has been observed and only a slight decrease in overall sensitivity was observed after the injection of numerous alka-

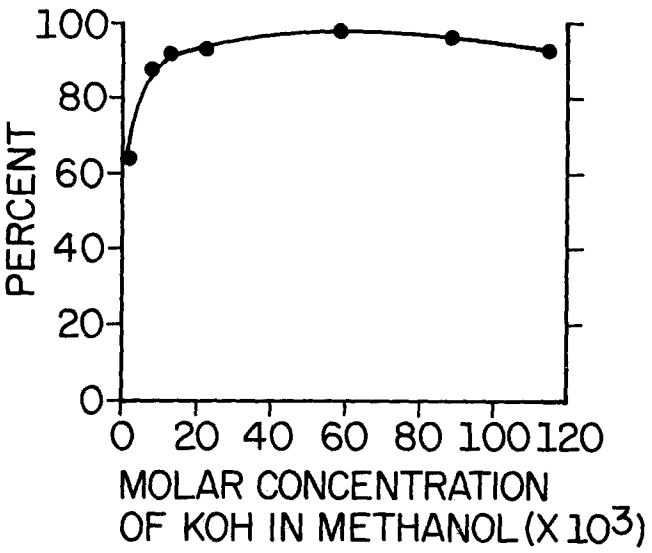

Figure 2. Efficiency of conversion of chlorphoxim to DEMTP in various concentrations of KOH in methanol.

line samples. This posed no real problem in that sensitivity was easily restored by simply replacing the silanized glass wool plug at the front of the column. As many as 270 samples have been analyzed with no decrease in sensitivity.

Preparation of samples

1) Water samples were extracted using essentially the same method reported by MILES et al. (1976) by placing 300 mL of the water to be analyzed in a 500-mL volumetric flask, adding 10 mL hexane, stirring 30 min with a magnetic stirrer, adding sufficient deionized water to bring the hexane layer into the neck of the flask, and removing a 5-mL aliquot. The 5-mL aliquot was transferred to a 15-mL centrifuge tube. The hexane was evaporated over a steam bath just to dryness and the last traces of vapor removed by horizontal rotation of the tube. An appropriate amount (0.5-5.0 mL) of 0.05 M KOH in methanol was added and the sample allowed to stand at room temperature overnight.

2) Fish samples. Bluegill fish weighing about 2 g each were prepared by cutting fish into small pieces and grinding with Na_2SO_4 in a mortar and pestle. Each sample was quantitatively transferred with acetone to a 250-mL Erlyenmeyer, screw-cap flask. The final volume of acetone was adjusted to about 100 mL and the samples placed on a Burrell Wrist-Action shaker for 30 min. Samples were filtered with suction through a glass fiber filter

and the flask and solid residue quantitatively rinsed with a small amount of acetone. The collected filtrate was evaporated over a steam bath to < 5 mL and transferred with no more than 20 mL acetone to a 500-mL volumetric flask containing 300 mL of deionized water. Ten mL of hexane was added to the 500-mL flask and the sample was extracted as described above for water. A 5-mL aliquot was transferred to a 15-mL centrifuge tube. The hexane was evaporated over a steam bath as described. A small amount of oily residue remained at this stage. To each sample an appropriate volume (0.5-5.0 mL) of 0.05 M KOH in methanol was added and the sample allowed to stand at room temperature overnight.

The amount of chlorphoxim contained in unknown samples was determined by a comparison of peak height responses obtained for the samples with that obtained for standard chlorphoxim in 0.05 M KOH in methanolic. The 0.05 M KOH in methanol must be added to unknown samples and standards at approximately the same time.

RESULTS AND DISCUSSION

Recovery of chlorphoxim in fish. Bluegill fish, which had not been exposed to any pesticide, were fortified with chlorphoxim at levels ranging from 0.05 to 22 ppm. The samples were extracted and analyzed without cleanup. Recovery of chlorphoxim averaged 83 ± 15% (Table 1).

TABLE 1

Recovery of Chlorphoxim from Fish Fortified at Various Concentration Levels

Chlorphoxim (ppm)		Percent
Added	Recovered	Recovery
0.052	0.050	96
0.102	0.101	99
0.504	0.403	80
1.03	0.677	66
10.1	7.33	73
14.8	12.1	82
21.6	17.9	83

$\bar{X}_7 = 83$, s = 12, CV = 15%

One of the most beneficial aspects of this method of conversion of chlorphoxim to DEMTP is that it eliminates any need for further cleanup of fish samples after extraction with hexane. As noted above, a small amount of oily residue does remain after evaporation of hexane. In some of the more heavily oiled samples a lack of homogeneity was observed immediately after addition of the methanolic KOH solution. The solution ususally cleared within

a few hours and no interference with the GLC analysis was experienced. Occasionally a precipitate resulted but this could be removed by transferring the methanolic KOH supernatant to another test tube after either allowing the precipitate to settle or centrifuging the sample at 1600 rpm for 10 min. Neither of these techniques interfered with chlorphoxim analysis.

Application of method to measure chlorphoxim residues in water and fish from treated aquaria. The method has been used to measure chlorphoxim residues in water and fish from aquaria treated with 20% emulsifiable concentrate of chlorphoxim. In a preliminary study, bluegill fish were exposed in an aquarium to a level of about 0.02 ppm chlorphoxim in water for 24 h. Analyses of the water and fish were performed using the methods described above. Results are shown in Table 2. This information is included here only to demonstrate the applicability of the method described, and no attempt will be made to explain any environmental significance which the data might have.

TABLE 2

Concentration of Chlorphoxim Found in Water and Bluegill Fish Exposed in Aquaria Treated With 0.02 ppm of Chlorphoxim Emulsifiable Concentrate

Tank	Chlorphoxim found, ppm		
	Water		Fish
	0 hours	24 hours	24 hours
1	0.022	0.015	2.93
2	0.025	0.016	2.77
3	0.023	0.018	3.05
$\bar{X}_3$	0.023	0.016	2.92
s	0.002	0.002	0.14
CV	6.6%	9.5%	4.8%

During gas chromatographic analysis of the above and subsequent samples, the unknown samples were compared to chlorphoxim standard prepared in 0.05 M KOH in methanol at the same time as the unknowns and to fresh dilutions of DEMTP in methanol. It has been verified that approximately 99% efficiency of conversion of chlorphoxim to DEMTP is maintained with the injection of actual biological samples.

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